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D9.124 – Characterisation of exosomes from control, irradiated and shielded tissues

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Abstract

Work performed within Task 3.1 was focused on "Characterization of exosomes from control irradiated and shielded tissues". This work has been completed for the mouse brain, liver and heart from whole body (WB) and partial body (PB) groups, 15 days and 24 hours post 2Gy irradiation as well as age-matching unirradiated controls (CN). Also, the functional assays have started using embryonic fibroblasts (MEFs). Interesting Initial results are showing increased DNA damage and increased adhesion to fibronectin in exosome-treated MEFs. Results for both parameters point to increased effects with 2Gy WBI and PBI liver exosomes treated MEF cells (OBU). The same functional assays are ongoing from the brain and heart exosomes and will be completed soon. This will facilitate the timely implementation of subsequent tasks. Finally, exosome samples from all experimental groups were shipped to each partner for experimental activities within their tasks.

D9.124 [D3 (Task 3.1)]: Characterization of exosomes from control irradiated and shielded tissues

OBU is involved in WP1 (Partial-body irradiations and dosimetry) and in WP3 (Radiation signalling between tissues).

Under WP1, at OBU two pilot experiments were conducted in order to optimize the exosome isolation methods. Brain and liver mouse organs were used with three different methods of exosome's isolation and analysis: ultracentrifugation, chemical/EV column, and Viva Spin Column. The results of these studies for the brain showed that the ultracentrifugation method resulted in the highest yield of exosomes (Fig. 1). Therefore, this extraction method was adopted for their characterization in tissues from control, irradiated and shielded mice.

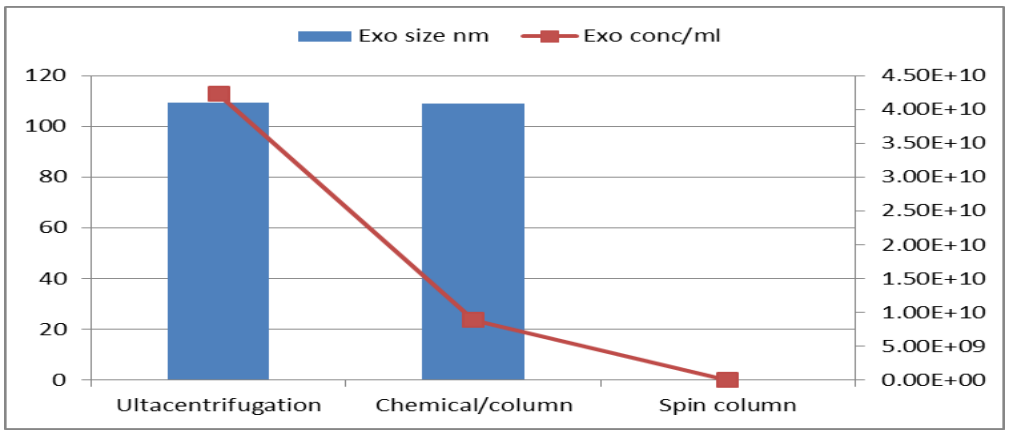

Fig. 1: Mouse brain. Data from the three different methods of exosome extraction.

We received further samples from ENEA that included brain, liver and heart collected from mice exposed to either whole-body (WBI) or partial-body Irradiation (PBI, shielded) with 2.0 Gy, or unirradiated (CN), and sacrificed 15 days after exposure. As shown in Table 1, the exosomes concentrations and analysis results showed: increased exosome concentrations in brain, liver and heart from irradiated mice (WBI and PBI) compared to controls (0 Gy). However, exosome concentrations in PBI organs was higher compared to WBI organs. The liver showed the highest yield of exosomes, while brain samples yield was moderate and the heart had the lowest yield (Liver > Brain > Heart). Exosomes samples from all the organs were shipped to each partner according to their request.

	Exosome concentration		
	0 Gy (CN)	2 Gy WBI	2 Gy PBI
Brain	1.20E+12/mL	2.83E+12/mL	4.27E+12/mL
Liver	8.70E+13/mL	1.07E+14/mL	1.20E+14/mL
Heart	3.70E+09/mL	5.27E+09/mL	1.57E+10/mL

Table 1. Exosome concentration/mL for all experimental groups.

With regards to the functional assay, after our initial experiments with mouse bone marrow cultures, embryonic fibroblasts (MEFs) were selected for this part of the WP task. Interesting initial results are showing increased DNA damage and increased adhesion to fibronectin in exosome-treated MEFs. Results for both parameters point to increased effects in MEF cells treated with 2Gy WBI and PBI liver exosomes (OBU).

Exosomes from brain and liver of WBI and PBI mice (0, 2 Gy, 15 days post exposure) have been characterised using Raman spectroscopy (TU Dublin, formerly DIT). Good clustering of the Raman spectral data into CN, PBI and TBI groups was observed for isolated exosomes from brain and liver.

To analyse shorter-term effects, exosomes were also isolated from brain, liver and heart collected from mice irradiated as above and sacrificed 24 hours after exposure. Similar to the samples collected from mice at 15 days post irradiation, the liver shows the highest yield of exosomes, while brain samples yield is moderate and the heart has the lowest yield of exosomes (Liver > Brain > Heart) (Table 2).

Exosome concentration/mL			
	0Gy (control)	2Gy WBI	2Gy PBI
Brain	2E+12	3.8E+12	4.1E+12
Liver	1.17E+13	2.83E+13	3.73E+13
Heart	5.95E+09	1.6E+10	1.74E+10

Table 2. Exosome concentration/mL for all experimental groups.
and heart following whole body and partial body post 24 hours
2Gy irradiation compared to 0Gy.

OBU has also started investigating exosomes characteristics from circulating blood, received by ENEA, at 24 h post irradiation following 0 Gy and 2 Gy WBI. Exosomes were extracted using ultracentrifugation and analysed for concentrations and size. The overall results show that plasma gives higher yield of exosomes than serum (Table 3). Exosomes have also been characterised using Raman spectroscopy (TU Dublin, formerly DIT). Analysis of the data is underway. The exosomes are also being analysed for the protein cargo at HMGU.

	Exosomes from plasma		Exosomes from serum	
	0 Gy	2 Gy	0 Gy	2 Gy
Exo concentration/mL	1.77E+09	1.43E+09	1.37E+09	8.7E+08
Exo diameter (nm)	100.6667	95.33333	97.66667	96.33333

Table 3: Exosomes characteristic analysis from mouse circulating blood at 24 hours post irradiation.